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SHORT COMMUNICATION

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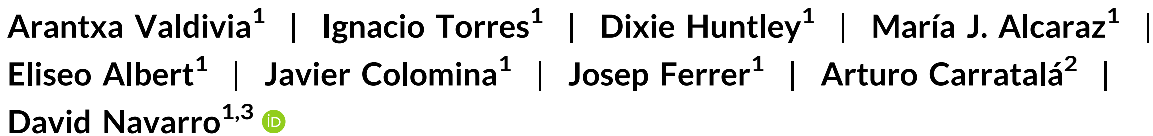
Qualitative assessment of SARS-CoV-2-specific antibody avidity by lateral flow immunochromatographic IgG/IgM antibody assay



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Abstract

Knowledge of the precise timing of SARS-CoV-2 infection may be of clinical and epidemiological relevance. The presence of low-avidity IgGs has conventionally been considered an indicator of recent infection. Here, we carried out qualitative assessment of SARS-CoV-2-specific antibody avidity using an urea (6M) dissociation test performed on a lateral flow immunochromatographic IgG/IgM device. We included a total of 76 serum specimens collected from 57 COVID-19 patients, of which 39 tested positive for both IgG and IgM and 37 only for IgG. Sera losing IgG reactivity after urea treatment (n = 28) were drawn significantly earlier ($P = .04$) after onset of symptoms than those which preserved it (n = 48). This assay may be helpful to estimate the time of acquisition of infection in patients with mild to severe COVID-19.

KEYWORDS

antibodies, avidity, Covid-19, SARS-CoV-2, urea dissociation

1 | INTRODUCTION

Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in respiratory tract specimens by reverse-transcription-based polymerase chain reaction (RT-PCR) assays is the mainstay of corona virus 2019 (COVID-19) diagnosis.[1] However, a nonnegligible fraction of COVID-19 patients test negative by RT-PCR on initial or consecutive upper respiratory tract specimens, due to a number of nonmutually exclusive preanalytical or analytical factors.[2] Although serology testing is mainly aimed at identifying individuals who have previously been exposed to SARS-CoV-2, it may also aid in diagnosis of ongoing COVID-19, particularly in RT-PCR negative patients who present at relatively late times after infection.[3] Knowledge of the precise timing of infection may be of clinical and epidemiological relevance as viral shedding in the upper respiratory tract seems to continue up to 7 to 9 days after onset of symptoms in patients presenting with mild or moderate COVID-19.[4-6] Often enough, however, this cannot be accurately determined. Theoretically, virus-specific serum IgM antibodies appear as soon as 7 days after infection and precede IgG seroconversion.[7] Nevertheless, both synchronous seroconversion of IgG and IgM, and IgM seroconversion occurring later than IgG have been documented in the setting of COVID-19,[8] casting doubt on the reliability of SARS-CoV-2 IgM as a biomarker of acute infection. Affinity maturation is a process by which Th_2-cell-activated B cells produce IgG antibodies with increased affinity for the antigen during the course of an immune response,[9] and avidity is defined as the combined affinities of a mixture of polyclonal IgG molecules.[10] Presence of low-avidity IgGs has conventionally been considered an indicator of recent infection.[10] Here, we carried out qualitative assessment of SARS-CoV-2-specific antibody avidity using an urea dissociation test performed on a lateral flow immunochromatographic device-lateral-flow immunochromatographic assay (LFIC),[11] and also discuss the potential clinical use of this approach.

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2 | PATIENTS AND METHODS

2.1 | Patients and sera

A total of 76 serum specimens collected from 57 COVID-19 patients were included in this study. Median age of patients (32 males and 25 females) was 66 years (range, 27-99 years). Forty-seven patients were admitted to our center with pneumonia, while the remaining 10 patients presented with mild symptoms not requiring hospitalization. Comorbid conditions including diabetes, cardiovascular diseases, chronic obstructive pulmonary disease or malignancies were identified in 47 patients. Clinical charts were reviewed to establish the time of onset of symptoms. The current study was approved by the Ethics Committee of Hospital Clínico Universitario INCLIVA.

2.2 | SARS-CoV-19 RT-PCR assays

Commercially-available SARS-CoV-2 RT-PCR assays used in the current study were detailed in previous publications.[12,13]

2.3 | SARS-CoV-2 antibody avidity assay

Qualitative assessment of SARS-CoV-2 antibody avidity was carried out using the LFIC ALLTEST 2019-nCoV IgG/IgM Rapid Test Cassette (Hangzhou ALLTEST Biotech Co., Ltd. Hangzhou, China), which uses a recombinant SARS-CoV-2 N protein as the antigen, following the manufacturer's recommendations.[14] Cryopreserved specimens (−80°C) were thawed and used for the experiments. A volume of 10 μL of serum was diluted into 1 mL of sample buffer before depositing (100 μL) into the appropriate location of the cassette (Test T-hole). When the fluid was about to reach the absorbent pad, 100 μL of sample buffer containing 6M urea was added to the T hole on the card. Serum specimens were run in parallel in the absence of urea treatment. Each reading was carried out independently by two observers after 20 minutes incubation. Appearance of either strong or weak sharp bands at the T line was recorded as a positive result. Absence of discernible lines was recorded as negative. Complete disappearance of reactive lines after urea treatment was interpreted as presence of low-avidity antibodies, whereas their persistence was taken to indicate high-avidity antibody presence.

2.4 | Statistical analysis

The Mann-Whitney U-test was used for comparison of medians. $P < .05$ were deemed to be statistically significant. The statistical package SPSS, version 21.0 (SPSS Inc) was employed.

3 | RESULTS AND DISCUSSION

Microbiological diagnosis of COVID-19 was made by RT-PCR in 44 patients, using RT-PCRs on upper respiratory tract specimens (URT), and by LFIC assay in the remaining 13 patients.

Out of the 76 sera, 39 tested positive for both IgG and IgM and 37 only for IgG. IgG+/IgM+ and IgG+/IgM− sera were obtained at a median of 20 days (range, 1-48 days) and 24 days (range, 6-59) after onset of symptoms, respectively ($P = .13$). In line with previous observations,[8] our data highlighted the wide variability in the kinetics of IgM and IgG detection across COVID-19 patients, which detracted from the reliability of IgM presence as a marker of acute infection.

Following urea treatment, IgG reactivity disappeared in 28 sera and persisted in 48. Sera losing IgG reactivity were obtained significantly earlier ($P = .04$) after onset of symptoms than those preserving it (median, 14.5 days; range, 1-45 days vs. median, 23 days; range, 5-59 days, respectively), although a certain degree of overlap was seen. IgG binding to SARS-CoV-2 after urea dissociation according to the time of sera collection is shown in Table 1.

Based upon the assumption that viable SARS-CoV-2 can be detected in URT specimens up to 9 days after symptoms onset,[4-6] we grouped sera into two categories according to whether they were drawn either before (n = 10) or after day 9 (n = 66) since symptoms onset. Low-avidity IgGs were detected in 8 out of the 10 former sera, and in 21 of the 66 latter sera ($P = .01$). Accordingly, the positive predictive value of the assay (IgG band) for correctly categorizing SARS-CoV-infection as an early one (<10 days after the onset of symptoms) was 80%, whereas the negative predictive value was 68%. Both predictive values were not adjusted to SARS-CoV-2 prevalence.

At least two consecutive sera were available from 15 patients (Table 2). Acquisition of high-avidity IgG antibodies in our system was clearly time-dependent, usually occurring 3 weeks after onset of symptoms, although in a few patients it could be documented earlier (patients 4, 5, and 15). This observation is in keeping with the idea that the dynamics of antibody affinity maturation varies across individuals.[7-10]

IgM reactivity was lost in 17 out of 39 sera after urea dissociation treatment, whereas it remained in 22. The time elapsed since

TABLE 1 Qualitative assessment of SARS-CoV-2-specific IgG antibody avidity in sera from patients with COVID-19 according to the time of specimen collection since the onset of symptoms

Time of serum collection, d	Number of sera tested/number of reactive sera after urea dissociation
1-7	5/1
8-14	20/10
15-21	10/9
22-28	19/11
>29	22/17

Abbreviations: COVID-19, corona virus 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 2 Qualitative assessment of SARS-CoV-2-specific antibody avidity in serial serum samples from patients with COVID-19

		Antibody reactivity			
Patient	Time since symptoms onset, d	IgG	IgG after urea treatment	IgM	IgM after urea treatment
1	27	+	−	−	−
	39	+	+	−	−
2	22	+	−	−	−
	34	+	−	−	−
3	25	+	−	+	−
	35	+	+	−	−
4	20	+	+	−	−
	24	+	+	−	−
5	10	+	−	+	+
	16	+	+	+	+
6	23	+	−	+	−
	24	+	−	+	−
7	30	+	+	−	−
	31	+	+	−	−
	51	+	+	−	−
	59	+	+	−	−
8	11	+	−	−	−
	23	+	+	−	−
9	30	+	+	+	−
	40	+	+	−	−
	47	+	+	−	−
10	7	+	−	+	+
	15	+	−	+	+
11	28	+	+	+	−
	40	+	+	+	−
12	21	+	−	−	−
	26	+	+	−	−
	38	+	+	−	−
13	21	+	+	+	+
	48	+	+	+	+
14	6	+	−	−	−
	14	+	−	−	−
15	14	+	+	+	−
	18	+	+	−	−

Abbreviations: COVID-19, corona virus 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

onset of symptoms did not differ across comparison groups (median 24 days; range 1-45 days vs 14.5 days; range 2-48 days, respectively; $P = .14$). Sera testing positive for rheumatoid factor (RF) IgM have been shown to yield false-positive IgM reactivity in a SARS-CoV-2 LFIC assay, and it has been reported that RF interference could be eliminated in most sera after urea dissociation.[11] This observation was validated in the current study: 5 out of 14 sera with leftover sample available that had lost IgM reactivity in the presence of urea tested positive for RF (median 18 IU/mL; range, 17 to 46 IU/mL; normal values <14 IU/mL). Collectively, these data suggest that SARS-CoV-2 N protein-reactive IgM avidity may vary across COVID-19 patients, regardless of the infection acquisition time; elucidation of whether this variability may impact on IgM functionality, and ultimately on COVID-19 prognosis could be an interesting focus of future research.

A total of nine sera from patients with seasonal human coronavirus infection occurring before the epidemic outbreak in our Health Department were also included in the current study. Coronaviruses were detected in URT specimens by a multiplex PCR assay (The NxTAG Respiratory Pathogen Panel; Luminex Corp, Austin, TX). Seven patients had coronavirus 229E and two patients had dual infections caused by coronavirus 229E and HKU1 and coronavirus 229E and NL63. Sera had been obtained at a median of 3 weeks after diagnosis. Only one of the nine sera tested IgG-positive, yet reactivity was lost following urea treatment. This suggested, but did not prove, that IgG antibodies targeting seasonal coronaviruses and cross-reacting with SARS-CoV-2 may display low avidity.

In summary, we adapted a commercially available LFIC IgG/IgM assay for qualitative assessment of SARS-CoV-2 antibody avidity that may be helpful to estimate the time of acquisition of infection in patients with mild to severe COVID-19. Our approach should be validated using conventional quantitative enzyme-linked immunosorbent assay or chemiluminiscent immunoassay avidity assays and also in cohorts including both asymptomatic and paucisymptomatic individuals. Further studies are warranted to elucidate how the kinetics of IgG avidity maturation correlates with that of virus excretion in the URT, to determine the extent to which contagiousness of COVID-19 patients can be inferred from absence of high-avidity IgGs and determine whether avidity of SARS-CoV-2-specific IgGs impact on clinical outcomes.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

AV, IT, DH, MJA, EA, JC, and JF performed serological and RT-PCR assays. AC carried out RF analyses. DN, AV, IT, and DH analyzed and interpreted the data. DN wrote the manuscript.

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